Literature Cited

(1) Danish, A. A., and Lidov, R. E., *Anal. Chem.*, **22,** 702 (1950).
(2) *Farm Chemicals*, **117,** No. 9, 26 (1954).
(3) Fleck, E. E., and Haller, H. L., *Ind. Eng. Chem.*, **37,** 403 (1945).
(4) Folckemer, F. B., and Loeffler, E. S., *Agr. Chemicals*, **9,** No. 2, 42 (1954).
(5) Jacob, K. D., J. AGR. FOOD CHEM., **2,** 970 (1954).
(6) O'Donnel, A. E., Neal, M. M., Weiss, F. T., Bann, J. M., De Cino, T. J., and Lau, S. C., *Ibid.*, **2,** 573 (1954).
(7) Shell Chem. Corp., Agl. Chem. Div., New York, N. Y., "Shell Method Series," Methods 523 and 572.

Received for review November 29, 1954. Accepted May 16, 1955. Presented at the 16th Midwest Regional Meeting, AMERICAN CHEMICAL SOCIETY, *Omaha, Neb., November 4 to 6, 1954.*

ANTIBIOTICS IN PLANT DISEASE

Comparative Studies on Control of Fireblight in Apple and Pear

H. F. WINTER and H. C. YOUNG
Ohio Agricultural Experiment Station, Wooster, Ohio

This work was undertaken in an attempt to provide an effective control for fireblight, a very damaging disease of pome fruits. The fact that the antibiotics used in medicine were largely effective against bacterial organisms led to their use for the control of fireblight, which is also caused by a bacterium. Laboratory tests showed that streptomycin and Terramycin were most effective in inhibiting the growth of the fireblight bacterium in vitro. Greenhouse studies indicated that foliage sprays of either material would largely prevent fireblight infection when young apple trees were inoculated by needle puncture with *Erwinia amylovora* 24 hours later. Extensive orchard experiments in 1953 and 1954 proved that fireblight of apple and pear can be effectively controlled by spraying during bloom with 50 to 100 p.p.m. of streptomycin. The development of an effective control for fireblight will greatly reduce the damage to apples and pears caused by this disease, and should allow the reestablishment of a profitable pear industry in the eastern United States.

LABORATORY SCREENING TESTS at the Ohio Agricultural Experiment Station during the winter and spring of 1952 indicated that certain antibiotic preparations developed for use in medicine were inhibitory to the fireblight pathogen, *Erwinia amylovora*. A serial dilution technique was used employing nutrient broth as the culture medium. Thiolutin, polymyxin, streptomycin, Chloromycetin, Terramycin, and several different types of penicillin were tried. Of those tested, Terramycin and streptomycin were inhibitory in the greatest degree.

Streptomycin, Terramycin, thiolutin, and polymyxin were next applied in several ways to young Jonathan trees in the greenhouse. Potted trees were watered with solutions of these antibiotics for 2 weeks and then inoculated with *Erwinia amylovora*. Other trees were treated by trunk injection; some were sprayed with antibiotic solutions.

As might have been expected, the antibiotics applied to the soil were either quickly inactivated or failed to be translocated to the susceptible tree parts in sufficient quantity to prevent infection. Results were erratic when tree trunks were injected with solutions of the antibiotics; some of the twigs inoculated on these trees became infected, while others remained healthy. The distribution of the antibiotics in the trees appeared to be very unequal. In addition, trees which were injected with solutions of 50 p.p.m. or more of any of the antibiotics used were severely injured.

When young, rapidly growing Jonathan apple trees in the greenhouse were sprayed with solutions of streptomycin sulfate or Terramycin hydrochloride and inoculated with *Erwinia amylovora* by needle puncture 24 hours later, fireblight infection was largely prevented. A foliage spray application of 100 p.p.m. of Terramycin or streptomycin gave 100% protection, when the sprayed and inoculated twigs were enclosed in a cellophane wrap; uncovered, 200 p.p.m. was required. It is probable that the moist condition of the sprayed foliage enclosed in the cellophane wrap facilitated the absorption of the antibiotic solutions. Since the inoculation was performed by needle puncture to the interior of succulent twigs, it was believed that considerable absorption or penetration of the antibiotics had taken place.

Chas. Pfizer & Co. furnished sufficient commercial grade streptomycin sulfate and crude Terramycin in the spring of 1953 to carry out an orchard experiment on bearing Jonathan apple trees. Three spray applications—early bloom, full bloom, and petal fall—of commercial grade streptomycin sulfate and crude Terramycin were made at dosages to give 60 and 120 p.p.m. of antibiotic activity. The crude Terramycin was first dissolved in methanol, as it was only slightly soluble in water. For comparison purposes, two "in bloom" spray applications of Dithane Z-78 at 2 pounds per 100 gallons of water were made to other trees in the block. Triton B-1956 at 2 ounces per 100 gallons of spray was added in all treatments to ensure adequate wetting and penetration of the sprays into the blossoms. To ensure the presence of adequate disease, all trees were inoculated by spraying them in full bloom with a suspension of *Erwinia amylovora* and pyrophyllite, according to the method suggested by Dunegan and others (*1*) for inoculating pear seedlings. All treatments were made to single trees replicated three times and randomized. Seventeen days after inoculation, abundant blossom cluster and twig blight was evident on the control trees and blight counts were made. The results are given in Table I.

Because of the very striking and favorable results in 1953 extensive experiments were planned and carried out during the 1954 season (*2, 3*), by essentially the same procedures as in 1953. These consisted of controlled experiments involving the inoculation of the test trees as well as the application of one or more formulations of streptomycin in 12 different commercial apple and pear orchards in all sections of Ohio. In the controlled tests, three different antibiotics were used—streptomycin, Terramycin, and tetracycline. Five different formulations of streptomycin were also tested. Table II gives the results obtained on Jonathan apple in three different orchards. A similar experiment was conducted on 4-year-old Bartlett pear. Pear trees inoculated but otherwise untreated showed an average of 60.2% of the twigs infected 3 weeks after inoculation. Pear trees sprayed three times with streptomycin sulfate at 100 p.p.m. activity showed only 0.37% twig infection.

Table I. Fireblight Studies, 1953

(In bloom sprays on inoculated Jonathan)

Materiel in 100 Gallons	% Blossom Cluster Blight, 5/25	% Twig Blight, 5/25
Streptomycin		
120 p.p.m.	2.0	0.0
60 p.p.m.	7.8	0.5
Terramycin		
120 p.p.m.	9.0	0.2
60 p.p.m.	20.6	5.2
Dithane Z-78, 2 lb.	44.0	9.8
Check, no fireblight sprays	83.1	20.6

Experiments in commercial orchards were a semidemonstration type. Growers were furnished one or more formulations of streptomycin weighed out in advance, so they needed only to add the contents of the bottle to the water-filled sprayer. Weighed quantities of Triton B-1956 were furnished, where this wetting agent was to be used. Complete written instructions for the application of the materials were also given the orchard operator. Generally, three spray applications were made in these tests—at early and full bloom, and at petal fall stages of bud development. The antibiotic sprays were always applied to rather large blocks of trees and in all cases similar control blocks were left untreated.

All orchards were visited at frequent intervals and when fireblight was well in evidence on the control trees, counts were made of the number of infections per tree. Table III gives the results obtained in five of the seven Jonathan apple orchards used in 1954. Insufficient blight for comparison purposes developed in the other two orchards.

Similar experiments were carried out in five commercial pear orchards in 1954. Fireblight developed in only two of these to a degree where significant data could be taken, and in one, the orchard operator cut out the blight infections before the orchard was visited. Hence, data were obtained from only one commercial pear orchard, a 6-year-old orchard at Dayton. Seventy-five untreated trees showed an average of 9.5 infections per tree on June 15. Ninety-five trees sprayed three times with Agrimycin at a dosage to give 100 p.p.m. streptomycin activity averaged only 0.42 infection per tree. In the pear orchard where the blighted material was removed prematurely, the orchard operator stated that only a few infections were removed from 150 Agrimycin-sprayed trees. On the other hand, a pickup truck load was removed from a similar number of untreated trees. It appears from these results and the results of the controlled test that streptomycin sprays will control fireblight on pear in about the same degree as on Jonathan apple.

Table III. Fireblight Control, 1954

(Commercial orchards, Jonathan apple)

Material Used	Infections per Tree: Baker orchard, 4-yr. trees	Moreland Hills orchard, 18-yr. trees[a]	Welday orchard, 15-yr. trees	Kampf orchard, 35-yr. trees	Lane orchard,[a] 18-yr. trees
Agrimycin	0.0	0.01	1.6	3.7	1.3
Streptomycin concd.	...	0.03	...	...	...
Streptomycin Sulfate	...	...	...	0.4	...
Streptomycin Sulfate[b]	...	...	2.3	...	...
Control (no treatment)	11.1	20.40	39.3	377.0	24.5

Natural infection, no inoculation.

Materials at dosages to give 100 p.p.m. of major antibiotic applied at early and full bloom, and at petal fall.

[a] Applied as 5× concentrate.

[b] Insoluble formulation of streptomycin sulfate.

Table II. Fireblight Control, 1954

(Jonathan apple trees inoculated in full bloom with *Erwinia amylovora*)

Material Used[a]	P.P.M.	Blight in Orchard 1, Wooster, % Blossom clusters	Blight in Orchard 1, Wooster, % Twigs	Blight in Orchard 2, Wooster, % Blossom clusters	Blight in Orchard 2, Wooster, % Twigs	Blight in Orchard 3, St. Clairesville, Blossom Clusters, %
Streptomycin sulfate[a]	50	...	...	5.7	0.3	9.5
Streptomycin sulfate[a]	100	1.2	0.0	3.2	0.1	2.9
Streptomycin nitrate[b]	100	7.7	0.0	7.4	0.2	...
Streptomycin concentrate[c]	100	0.5	0.0	10.1	0.7	...
Agrimycin[c]	100	1.8	0.0	...	...	...
Terramycin hydrochloride[c]	100	24.8	2.0	21.2	2.9	30.4
Tetracycline[c]	100	24.5	6.1	22.7	9.0	...
Control	None	62.2	23.2	56.9	29.3	90.6

[a] Dosages given are on basis of antibiotic activity of major antibiotic in formulation. Triton B-1956 at 2 ounces per 100 gallons used in all treatments except Agrimycin.

[a] Commercial grade furnished by Merck & Co.

[b] Furnished by Mathieson Chemical Co.

[c] Furnished by Chas. Pfizer & Co.

Summary

The control of fireblight of apples and pears by streptomycin sprays has been excellent in Ohio over a 2-year period, both in controlled experiments involving the inoculation of trees and in extensive tests in commercial orchards. In no case, where three streptomycin sprays were employed at a dosage to give 100 p.p.m. activity, was there failure to control fireblight. Five different antibiotic formulations containing streptomycin gave significant control. Terramycin and tetracycline also gave a significant control of fireblight, but were inferior to formulations containing streptomycin. Agrimycin, a trade-named agricultural antibiotic formulation containing streptomycin and Terramycin in a 10 to 1 ratio, gave results equal to but not superior to those obtained with streptomycin alone.

Literature Cited

(1) Dunegan, J. C., Moon, H. H., and Wilson, R. A., *Plant Disease Reptr.*, **37**, 14 (1953).

(2) Winter, H. F., and Young, H. C., *Phytopathol.*, **44**, 511 (1954) (abstract).

(3) Winter, H. F., and Young, H. C., *Plant Disease Reptr.*, **37**, 463 (1953).

Received for review September 13, 1954. Accepted May 2, 1955. Presented before the American Phytopathological Society, Estes Park, Colo., August 1954.